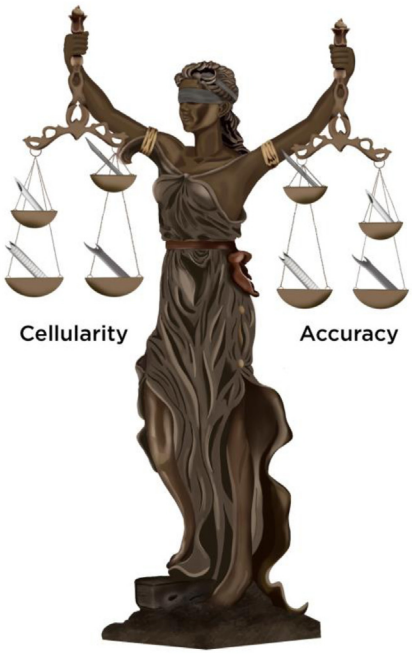
Clinical Gastroenterology and Hepatology 2020;■:■–■

Comparing Needles and Methods of Endoscopic Ultrasound–Guided Fine-Needle Biopsy to Optimize Specimen Quality and Diagnostic Accuracy for Patients With Pancreatic Masses in a Randomized Trial

Q16 Ji Young Bang,* Konrad Krall,‡ Nirag Jhala,§ Charanjeet Singh,‡ Mohamedtaki Tejani,‖ Juan Pablo Arnoletti,¶ Udayakumar Navaneethan,* Robert Hawes,* and Shyam Varadarajulu*

*Center for Interventional Endoscopy, ‡Department of Pathology, AdventHealth Orlando, Orlando, Florida; §Department of Pathology and Laboratory Medicine, Temple University Hospital, Philadelphia, Pennsylvania; ‖Department of Medical Oncology, ¶Center for Surgical Oncology, AdventHealth Cancer Institute, Orlando, Florida



Clinical Gastroenterology and Hepatology

Q6 **BACKGROUND & AIMS:** Given the lack of procedure standardization, findings vary from analyses of pancreatic tissues collected by endoscopic ultrasound–guided fine-needle biopsy. It is not clear which needle and technique yield the best specimen for analysis. We compared the specimen quality and accuracy of diagnoses made from samples collected by fine-needle biopsy needles using different collection techniques.

METHODS: Patients found to have pancreatic masses during imaging (n = 129) were assigned randomly to groups from whom pancreatic tissue samples were collected by reverse-bevel, Menghini-tip, franseen, or fork-tip needles. A second randomization determined the technical sequence of biopsies in each patient (suction, no suction, and stylet retraction). Two independent pathol-

Abbreviations used in this paper: EUS-FNA, endoscopic ultrasound–guided fine-needle aspiration; FNB, fine-needle biopsy; ROSE, _____.

1542-3565/$36.00
https://doi.org/10.1016/j.cgh.2020.06.042

ogists, blinded to the type of needle and sampling technique, analyzed all the samples. Final diagnoses of malignancy were made based on surgical resection, death from cancer progression, or findings from radiology or clinical follow-up evaluations (reference standard). The primary objective was to compare the cellularity of the samples collected, defined as the proportion of core tissue in the biopsy sample. Secondary objectives were to compare the accuracy of diagnoses made from biopsy samples and identify factors associated with high cellularity.

RESULTS: **One-hundred and nine patients had a final diagnosis of malignancy (84.5%) and 20 patients had benign disease (15.5%). Samples collected by fork-tip or franseen needles had significantly higher cellularity than samples collected by reverse-bevels or Menghini-tip needles ($P < .001$). Neoplasias were identified with greater than 90% accuracy using samples collected by fork-tip or franseen needles ($P < .001$ compared with other needles). On multivariable regression analysis, use of franseen needles (odds ratio [OR], 4.42; 95% CI, 2.58–7.58; $P < .001$) or fork-tip needles (OR, 3.86; 95% CI, 2.24–6.64; $P < .001$), stylet retraction (OR, 2.13; 95% CI, 1.21–3.72; $P = .008$), no suction (OR, 2.74; 95% CI, 1.57–4.80; $P < .001$), and pancreatic mass larger than 3 cm (OR, 1.92; 95% CI, 1.21–3.05; $P = .005$) were associated with high cellularity of the sample.**

CONCLUSIONS: **In patients with suspected pancreatic cancer, samples with the highest degree of cellularity in a single biopsy, resulting in a diagnostic accuracy of 90% of higher, were collected by fine-needle biopsy using the franseen or fork-tip needle. Clinicaltrials.gov no: NCT04085055.**

Keywords: EUS; FNB; Tumor; Sample Quality.

Q7 Endoscopic ultrasound–guided fine-needle aspiration (EUS-FNA) is integral to the diagnosis and Q8 staging of gastrointestinal malignancies, particularly pancreatic tumors. Although several steps are involved in determining the overall procedural outcomes, one key factor is the technique adopted for tissue procurement, which includes applying suction or retracting the needle stylet during tissue sampling.[1–3] These variations in techniques, however, contribute to poor procedural standardization and thereby the wide variation in reported outcomes for the diagnostic sensitivity of EUS-FNA in pancreatic cancer, ranging from 50% to 100%.[4]

Cytologic aspirates from EUS-FNA have limited diagnostic sensitivity in chronic pancreatitis and often with suboptimal cellularity. Fine-needle biopsy (FNB) devices with unique geometries have been developed to procure specimens showing tissue architecture.[5] Given promising data that a histologic yield of more than 90% can be accomplished,[6,7] recent studies recommend that FNB needles be used preferentially, in lieu of FNA, for sampling all solid mass lesions identified at EUS.[8] This is particularly important because EUS-FNB is becoming an essential tool for the molecular and genetic characterization of peri-ampullary carcinomas to guide individualized and targeted cancer therapies. Unfortunately, there is discrepancy in the literature on the reported tissue yield of FNB devices, ranging from 59% to 95%.[6,9–11] It is unclear if this is related to the type of FNB needle being used or the technique adopted for sampling.

Although the National Comprehensive Cancer Network recommends EUS guidance to be the preferred method for tissue acquisition in patients with suspected pancreatic cancer,[12] the procedure is not widely available in rural and nonteaching hospitals, where the use of percutaneous biopsies still is prevalent.[13] One major impediment to the wider adoption of EUS is variability in outcomes, which likely is related to poor standardization of the procedure.

Given the increased use of EUS-guided FNB as the preferred strategy for tissue acquisition in suspected pancreatic cancer, it is important to find an answer to one elusive but sentinel question: which needle and technique likely will yield the most optimal specimen, preferably in a single pass? The answer to this critical question will simplify the procedure and enable standardization of outcomes. To test this concept, we designed a 4-arm randomized trial correlating needle design and procedural technique with findings at pathology and clinical follow-up evaluation. The study hypothesis was that, given the unique geometry, tailoring the sampling technique to specific needle design likely will yield the most optimal biopsy specimen. The primary end point therefore was to compare the cellularity for all available Federal Drug Administration–approved FNB needles. The secondary end points were to compare operating characteristics of biopsy samples and to identify factors associated with cellularity.

Materials and Methods

Patients and Settings

This randomized trial was conducted at AdventHealth Orlando (Orlando, FL). All patients aged 18 years and older with suspected solid pancreatic mass lesions that were identified on computed tomography scan or magnetic resonance imaging and referred for EUS-FNB were eligible for participation. Patients were excluded if a pancreatic mass lesion was not seen at EUS, the mass had a cystic component, or if coagulation parameters were abnormal. Written informed consent was obtained from all patients.

All authors had access to the study data and reviewed and approved the final manuscript.

Randomization and Masking

Computer-generated randomization assignments were obtained using the block randomization method and placed in sequentially numbered, sealed, opaque envelopes to be opened by the endoscopy nurse intraprocedurally when patients met inclusion criteria. Patients were randomized equally to 1 of the 4 FNB (22G) needle cohorts (1:1:1:1 allocation): reverse-bevel, Menghini-tip, franseen, or fork-tip. A subsequent randomization assignment in the same envelope determined the sequence in which sampling was performed using the 3 most commonly described techniques: application of negative suction, not applying any suction, or slow retraction of the stylet.

Two independent pathologists with expertise in gastrointestinal diseases, blinded to the type of needle and sampling technique, rendered interpretations.

Procedural Technique and Tissue Analysis

All procedures were performed using a linear array echoendoscope (Olympus GF-UCT180; Olympus America, Inc, Center Valley, PA) after administration of propofol using 1 of 4 FNB (22G) needles (ProCore; Cook Endoscopy, Winston-Salem, NC; EZ Shot 3 Plus; Olympus America, Inc; Acquire; Boston Scientific Corporation, Marlborough, MA; or SharkCore; Medtronic, Sunnyvale, CA) by 1 of 4 experienced endosonographers (>750 EUS procedures/y). The 4 needle-tip designs are shown in Supplementary Figure 1 and their geometries are described in the Supplementary Appendix. During EUS-FNB, the stylet was removed after puncturing the pancreatic mass, and sampled using the fanning technique (4 strokes at 4 locations within the mass) (Supplementary Video 1). Details of individual sampling techniques are included in the Supplementary Appendix. Three dedicated passes were performed using the assigned needle: 1 pass for each of the 3 techniques, with the order of the technique determined by the randomization sequence. The tissue specimens from each pass were collected separately in 10% formalin for tissue analysis.

Details on the method of tissue preparation and histologic assessment are included in the Supplementary Appendix. To limit subjective interpretation, specimens were quantified using specialized image analyzing software (Nikon DS-Fi2 color camera and NIS-Elements Basic Research Software Version 4.5; Nikon Instruments, Inc, Melville, NY) that measured the total specimen area, core tissue (acinar and ductal cells, fibrosis, and tumor when applicable), blood, and crush artifact.[6,7] This basic research imaging software has been used previously in other medical specialties to evaluate and quantify tissue morphology.[14–16]

Immediate adverse events were documented at the time of the procedure and late adverse events by telephone follow-up evaluation at 7 and 30 days after the procedure.

Definitions

The primary outcome, cellularity, was defined as the proportion of core tissue to the total specimen area, the grading of which was determined by previously established criteria (Supplementary Appendix). The core tissue sample comprised predominantly either acinar and ductal cells, fibrosis, and tumor cells (when applicable). The diagnostic adequacy of the tissue core was defined as the presence of pancreatic parenchyma and tumor (when applicable). Nondiagnostic tissue was defined as suboptimal or insufficient material that was not conducive for interpretation or representative of the final diagnosis. Technical failure was defined as needle malfunction before the 3 sampling maneuvers could be

What You Need to Know

Background

Given the lack of procedure standardization, findings vary from analyses of pancreatic tissues collected by endoscopic ultrasound–guided fine-needle biopsy. It is not clear which needle and technique yield the best specimen for analysis

Findings

In patients with suspected pancreatic cancer, samples with the highest degree of cellularity in a single biopsy, resulting in a diagnostic accuracy of 90% of higher, were collected by fine-needle biopsy using the Franseen or fork-tip needle.

Implications for patient care

Samples should be collected from patients with pancreatic masses by fine-needle biopsy using the Franseen or fork-tip needle, to increase the quality of the sample and accuracy of analysis.

performed. Adverse events were defined according to a predefined consensus.[17]

A final diagnosis of malignancy was defined by 1 or more of the following criteria: (1) histologic evidence of malignancy in the current sample or subsequent surgical resection specimen, (2) progression of the lesion or the presence of metastases on follow-up imaging, (3) cancer-related death, and (4) follow-up evaluation with the patient's referring physician confirming death or disease progression as a result of pancreatic cancer. Lesions were considered benign if they met 1 or more of the following criteria: (1) surgical pathology reported no malignancy, (2) follow-up imaging at 6 months reported a resolved or stable mass with no increase in size or metastases, and (3) patient well-being at the 6-month follow-up evaluation with the primary care physician. The reference standard for classification of disease included the following: surgical resection, death from disease progression, repeat radiologic, and/or clinical follow-up evaluation.

Outcome Measures

The primary outcome measure was to compare the degree of cellularity in biopsy samples obtained from EUS-FNB of pancreatic masses between the 4 needle types for a single pass, adopting the 3 sampling techniques. Secondary outcomes were to compare operating characteristics (sensitivity, specificity, positive predictive value, negative predictive value, and accuracy) of FNB

Table 1. Comparison of Patient and Mass Characteristics and Procedure Details According to Needle Type

	Reverse-bevel (n = 33)	Menghini-tip (n = 33)	Franseen (n = 32)	Fork-tip (n = 31)	*P* value
Age, *y*					
Mean (SD)	71.9 (10.6)	67.9 (13.8)	69.8 (9.9)	63.8 (15.5)	
Median	74	71	72	64	.054
IQR	67–79	61–78	64.5–77	54–71	
Range	50–89	28–94	46–88	25–92	
Sex, n (%)					
Female	16 (48.5)	13 (39.4)	18 (56.3)	14 (45.2)	.587
Male	17 (51.5)	20 (60.6)	14 (43.7)	17 (54.8)	
Race, n (%)					
Black	4 (12.1)	1 (3.0)	4 (12.5)	2 (6.5)	.370
White	28 (84.8)	27 (81.8)	24 (75.0)	23 (74.2)	
Other[a]	1 (3.0)	5 (15.2)	4 (12.5)	6 (19.4)	
Mass size, *cm*					
Mean (SD)	3.0 (0.9)	3.1 (1.0)	3.2 (1.1)	3.1 (1.1)	
Median	3.0	3.0	3.0	3.0	.986
IQR	2.5–3.6	2.7–3.4	2.5–4.0	2.0–4.0	
Range	1.2–5.0	1.0–6.0	0.6–6.0	1.0–6.0	
Mass location, n (%)					
Head/uncinate/genu	25 (75.8)	27 (81.8)	24 (75.0)	23 (74.2)	.882
Body/tail	8 (24.2)	6 (18.2)	8 (25.0)	8 (25.8)	
Vascular invasion, n (%)[b]	13 (39.4)	14 (42.4)	17 (53.1)	15 (48.4)	.689
Distant metastases, n (%)[c]	6 (18.2)	6 (18.2)	3 (9.4)	5 (16.1)	.732
Route of EUS-FNB, n (%)					
Transgastric	8 (24.2)	6 (18.2)	8 (25.0)	8 (25.8)	.882
Transduodenal	25 (75.8)	27 (81.8)	24 (75.0)	23 (74.2)	
Type of mass, n (%)[d]					
Malignancy	19 (57.6)	26 (78.8)	26 (81.3)	22 (71.0)	.284
Other neoplasia	7 (21.2)	4 (12.1)	1 (3.1)	4 (12.9)	
Benign	7 (21.2)	3 (9.1)	5 (15.6)	5 (16.1)	
Needle dysfunction, n (%)[e]	4 (12.1)	0	0	1 (3.2)	.034
Adverse events, n (%)[f]	1 (3.0)	1 (3.0)	2 (6.3)	1 (3.2)	.886

EUS-FNB, endoscopic ultrasound–guided fine-needle biopsy; IQR, interquartile range.
[a]Other includes 2 Asians and 14 Hispanic patients.
[b]Vascular invasion involved an artery in 11 patients, a vein in 43 patients, and both an artery and a vein in 5 patients.
[c]Metastases to the liver in 16 patients, brain in 1 patient, lungs in 2 patients, and peritoneum in 1 patient.
[d]Final diagnosis: benign disease in 20 patients (all chronic pancreatitis); malignancy in 93 patients (92 pancreatic adenocarcinoma, 1 squamous cell carcinoma); and other neoplastic lesions in 16 patients (1 gastrointestinal stromal tumor, 5 lymphoma, 7 neuroendocrine tumor, 3 solid pseudopapillary tumor).
[e]Needle dysfunction: reverse-bevel: broken or bent needle during FNB in 3 patients and needle did not exit the sheath during FNB in 1 patient; and fork-tip: bent needle during FNB in 1 patient.
[f]Adverse events: reverse-bevel: postprocedural abdominal pain, requiring admission for observation for 1 day in 1 patient; Menghini-tip: upper gastrointestinal bleed from a malignant duodenal ulcer in 1 patient; franseen: self-limiting mucosal bleeding during FNB in 1 patient, upper gastrointestinal bleed 2 days after FNB, which stopped with conservative management in 1 patient; fork-tip: postprocedural abdominal pain, requiring an emergency room visit in 1 patient.

samples for a single pass, based on the final diagnosis as defined earlier. Other outcomes included diagnostic adequacy, specimen bloodiness and crush artifact in the biopsy sample, diagnostic sensitivity for detecting cancer, predictors of high cellularity in the biopsy sample, technical failure, and adverse events.

Sample Size Calculation

The sample size calculation was based on the primary outcome measure of the procurement of high cellularity biopsy samples. A 2-tailed sample size calculation was performed with a type I error of 0.005 at 80% power with the estimated rate of procurement of a high cellularity sample of 25% using the reverse-bevel needle, compared with the rate of procurement of a high cellularity sample of 65% using the newer-generation Menghini-tip, franseen, and fork-tip needles.[6,7,18–22] This resulted in a sample size of 28 patients per group (total sample size, 112 patients) and hence was set at 31 patients per group to account for a 10% drop-out rate.

Statistical Analysis

Baseline patient demographics, characteristics of pancreatic mass lesions, procedural details, and outcomes were summarized as means (with SD) and medians (with interquartile range and range) for continuous data and as frequencies and proportions for categoric data. Continuous data were compared using the Kruskal–Wallis test and categoric data were compared using the chi-square test or the Fisher exact test as indicated. Operating characteristics of biopsy samples also were calculated for each needle type and technique and were compared using the chi-square test.

To identify factors associated with high cellularity on biopsy samples for all mass lesions and for pancreatic neoplastic lesions, multiple logistic regression and reverse stepwise multivariate logistic regression analyses were performed. All relevant clinical and procedural variables were used, including patient demographics, mass characteristics, needle type, and sampling technique.

Table 2. Procedure Outcomes for Each Technique for a Single Pass, With Comparison Between the Needles

	Reverse-bevel (n = 33)	Menghini-tip (n = 33)	Franseen (n = 32)	Fork-tip (n = 31)	*P* value[a]
Technique: no suction					
Diagnostic adequacy, n (%)	21 (63.6)	22 (66.7)	30 (93.8)	29 (93.5)	.001
Cellularity, %					
Mean (SD)	25.8 (33.8)	31.6 (40.8)	63.1 (36.9)	74.6 (36.8)	
Median	9.3	8.1	70.3	96.5	<.001
IQR	0–41.0	0–77.8	36.9–99.4	48.5–100	
Range	0–100	0–100	0–100	0–100	
Low	17 (51.5)	18 (54.5)	5 (15.6)	3 (9.7)	<.001
Intermediate	8 (24.2)	5 (15.2)	7 (21.9)	5 (16.1)	
High	8 (24.2)	10 (30.3)	20 (62.5)	23 (74.2)	
Crush artifact present, n (%)	2 (6.1)	2 (6.1)	6 (18.8)	5 (16.1)	.243
Technique: stylet retraction					
Diagnostic adequacy, n (%)	27 (81.8)	29 (87.9)	32 (100)	31 (100)	.011
Cellularity, %					
Mean (SD)	31.5 (31.7)	34.7 (31.9)	66.6 (30.9)	61.0 (31.6)	
Median	22.4	30.3	73.1	55.5	<.001
IQR	6.8–38.4	4.1–49.5	40.5–98.8	33.7–99.7	
Range	0–100	0–100	5.0–100	4.0–100	
Low	10 (30.3)	10 (30.3)	2 (6.2)	2 (6.5)	<.001
Intermediate	16 (48.5)	15 (45.5)	8 (25.0)	12 (38.7)	
High	7 (21.2)	8 (24.2)	22 (68.8)	17 (54.8)	
Crush artifact present, n (%)	3 (9.1)	1 (3.0)	10 (31.3)	5 (16.1)	.010
Technique: suction					
Diagnostic adequacy, n (%)	28 (84.8)	33 (100)	32 (100)	31 (100)	.002
Cellularity, %					
Mean (SD)	31.2 (32.4)	29.5 (24.8)	40.5 (31.3)	43.3 (30.8)	
Median	23.4	23.2	34.5	41.3	.140
IQR	7.2–50.3	10.9–40.9	9.6–61.5	16.1–66.4	
Range	0–100	0.98–93.8	0.069–99.9	0.28–100	
Low	13 (39.4)	8 (24.2)	9 (28.1)	5 (16.1)	.196
Intermediate	12 (36.4)	20 (60.6)	11 (34.4)	16 (51.6)	
High	8 (24.2)	5 (15.2)	12 (37.5)	10 (32.3)	
Crush artifact present, n (%)	5 (15.2)	5 (15.2)	8 (25.0)	4 (12.9)	.578

IQR, interquartile range.
[a]For comparison of degree of cellularity, the *P* value is for comparison of low-/intermediate-cellularity vs high-cellularity biopsy samples.

Statistical significance was determined at a *P* value less than .05. Analyses of the outcome measures and reporting were performed using the intention-to-treat method. All statistical analyses were performed using Stata 14 (Stata Corp, College Station, TX). The results are reported in accordance with the Consolidated Standards of Reporting Trials 2010 guidelines and the Standards for Reporting of Diagnostic Accuracy guideline.

Results

Of 172 consecutive patients screened for participation in this study between September 2019 and December 2019, 43 patients were excluded (Supplementary Figure 2). A total of 129 patients constituted the study cohort and were randomized to 1 of 4 groups.

Patient Demographics, Tumor Characteristics, and Procedure Details

There was no significant difference in patient demographics, tumor characteristics, or postprocedure adverse events between the cohorts (Table 1). A final diagnosis of carcinoma was established in 93 patients (72.1%), other neoplasia in 16 patients (12.4%), and benign disease in 20 patients (15.5%). Of 109 patients with neoplastic disease, 15 patients underwent surgical resection, 71 were undergoing chemotherapy with or without radiation therapy, 19 were deceased secondary to underlying malignancy, and 4 elected not to receive any treatment and had clinical–radiologic evidence of disease progression (metastases). Of the 20 patients with benign disease, 2 underwent repeat EUS-FNB with a confirmation of benign disease and 18 were asymptomatic at a median follow-up period of 7 months, with resolution or a decrease in size of mass on computed tomography imaging.

Biopsy Samples

Primary outcome: cellularity for a single pass. Cellularity was significantly higher for the franseen and fork-tip needles compared with the Menghini-tip and reverse-bevel needles, for both the no suction and stylet retraction techniques (Table 2, Figure 1, and Supplementary Figure 3). The median cellularity was suboptimal for all 4 needles when applying suction. Bloodiness in the biopsy sample was significantly lower

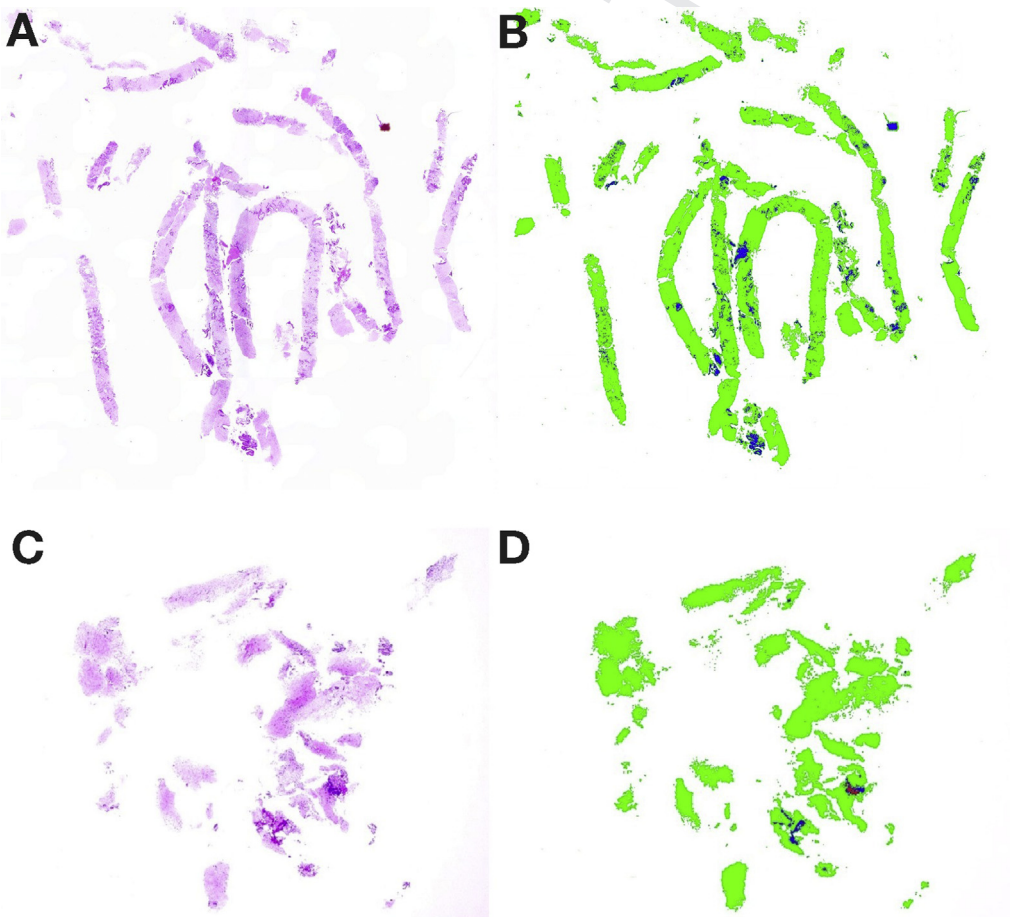


Figure 1. Images showing H&E staining (*left panels*) of pancreatic adenocarcinoma (20×) with corresponding digital images (*right panels*) showing adenocarcinoma with pancreatic acinar and ductal cells, fibrosis, blood, and crush artifact for (*A* and *B*) franseen and (*C* and *D*) fork-tip needles. In the digital images, core tissue is highlighted in green, blood is highlighted in blue, and crush artifact is highlighted in red.

Table 3. Multiple Logistic Regression Analysis Examining the factors Associated With Obtaining High Cellularity in Biopsy Samples During EUS-FNB

	Variable		Odds ratio	95% CI	*P* value
All lesions	Multiple logistic regression analysis				
	Needle type	Menghini-tip vs reverse-bevel	1.08	0.55–2.15	.816
		Franseen vs reverse-bevel	4.72	2.47–9.03	<.001
		Fork-tip vs reverse-bevel	4.41	2.27–8.58	<.001
	Technique	Stylet retraction vs suction	2.14	1.22–3.76	.008
		No suction vs suction	2.77	1.58–4.86	<.001
	Age, *y*		1.01	0.99–1.03	.159
	Sex, male vs female		1.07	0.68–1.70	.762
	Race, white vs other		1.13	0.65–1.94	.669
	Mass size, >3 vs ≤3 cm		1.87	1.16–3.02	.011
	Mass location, body/tail vs head/uncinate/genu		1.50	0.87–2.60	.146
	Reverse stepwise multivariate logistic regression analysis				
	Needle type	Franseen vs reverse-bevel	4.42	2.58–7.58	<.001
		Fork-tip vs reverse-bevel	3.86	2.24–6.64	<.001
	Technique	Stylet retraction vs suction	2.13	1.21–3.72	.008
		No suction vs suction	2.74	1.57–4.80	<.001
	Mass size, >3 vs ≤3 cm		1.92	1.21–3.05	.005
Pancreatic neoplastic lesions only	Multiple logistic regression analysis				
	Needle type	Menghini-tip vs reverse-bevel	0.97	0.47–2.03	.940
		Franseen vs reverse-bevel	5.18	2.53–10.6	<.001
		Fork-tip vs reverse-bevel	4.43	2.14–9.17	<.001
	Technique	Stylet retraction vs suction	2.13	1.16–3.93	.015
		No suction vs suction	3.16	1.71–5.84	<.001
	Age, *y*		1.01	0.99–1.03	.275
	Sex, male vs female		0.97	0.59–1.60	.908
	Race, white vs other		1.22	0.68–2.19	.508
	Mass size, >3 vs ≤3 cm		1.52	0.90–2.58	.118
	Mass location, body/tail vs head/uncinate/genu		1.78	0.96–3.31	.067
	Reverse stepwise multivariate logistic regression analysis				
	Needle type	Franseen vs reverse-bevel	5.22	2.89–9.43	<.001
		Fork-tip vs reverse-bevel	4.24	2.34–7.65	<.001
	Technique	Stylet retraction vs suction	2.11	1.15–3.88	.016
		No suction vs suction	3.12	1.69–5.74	<.001
	Mass location: body/tail vs head/uncinate/genu		1.88	1.06–3.32	.031

EUS-FNB, endoscopic ultrasound–guided fine-needle biopsy.

for the franseen and fork-tip needles compared with the Menghini-tip and reverse-bevel needles, for both the no suction and stylet retraction techniques. Bloodiness was high when using suction for all 4 needles. There was no significant difference in the presence or quantity of crush artifact irrespective of the needle or sampling technique used (Supplementary Tables 1 and 2).

Diagnostic adequacy for a single pass. The diagnostic adequacy was significantly higher at 93.8% to 100% for the franseen and fork-tip needles compared with 63.6% to 87.9% for the Menghini-tip and reverse-bevel needles for both the no suction and stylet retraction techniques (Table 2). The diagnostic adequacy was significantly higher at 100% when applying suction, for all needles except the reverse-bevel needle (84.8%). Immunohistochemistry studies were requested in 36 subjects and there was adequate tissue to perform testing successfully in all patients. Molecular profiling was requested in 6 patients, 2 in the franseen, 2 in the fork-tip, and 1 each in the Menghini-tip and reverse-bevel cohorts, and the biopsy sample was sufficient for analysis in all 6 subjects.

Predictors of high cellularity for pancreatic mass lesions. On reverse-stepwise multivariate logistic regression analysis, the use of the franseen needle, the fork-tip needle, not applying suction, use of stylet retraction, and pancreatic mass greater than 3 cm in size were associated with high cellularity (Table 3).

Operating characteristics. Although not the primary outcome of our study, we calculated operating characteristics for biopsy samples procured using the FNB needles. The overall sensitivity and accuracy of the franseen and fork-tip needles for diagnostic performance were 90% or greater and were significantly higher than the Menghini-tip and reverse-bevel needles (Table 4). However, the diagnostic sensitivity and accuracy for the Menghini-tip needle improved to 86.7% and 87.9%, respectively, when applying suction.

Pancreatic neoplasia and predictors of high cellularity in neoplasia. The diagnostic sensitivity of the franseen and fork-tip needles for diagnosing neoplasia was significantly higher and ranged from 92.6% to 100% for all 3 techniques, compared with the maximum

Table 4. Diagnostic Performance of Biopsy Samples for Each Technique, With Comparison Between the Needles

Technique	Operating characteristic	Needle type: Reverse-bevel	Menghini-tip	Franseen	Fork-tip	*P* value
All lesions						
All techniques	Sensitivity, % (95% CI)	61.0 (49.6–71.6)	72.8 (62.6–81.6)	91.6 (83.4–96.5)	96.3 (89.4–99.2)	<.001
	Specificity, % (95% CI)	100 (80.5–100)	100 (59.0–100)	100 (75.3–100)	100 (75.3–100)	.999
	PPV, % (95% CI)	100 (92.9–100)	100 (94.6–100)	100 (95.3–100)	100 (95.3–100)	.999
	NPV, % (95% CI)	34.7 (21.7–49.6)	21.9 (9.3–40.0)	65.0 (40.8–84.6)	81.3 (54.4–96.0)	<.001
	Accuracy, % (95% CI)	67.7 (57.5–76.7)	74.7 (65.0–82.9)	92.7 (85.6–97.0)	96.8 (90.9–99.3)	<.001
No suction	Sensitivity, % (95% CI)	48.3 (29.4–67.5)	56.3 (37.7–73.6)	90.0 (72.6–97.8)	90.0 (71.8–97.7)	<.001
	Specificity, % (95% CI)	100 (39.8–100)	100 (2.5–100)	100 (29.2–100)	100 (29.2–100)	.999
	PPV, % (95% CI)	100 (76.8–100)	100 (81.5–100)	100 (86.8–100)	100 (86.3–100)	.999
	NPV, % (95% CI)	21.1 (6.1–45.6)	6.7 (0.2 - 31.9)	50.0 (11.8–88.2)	50.0 (11.8–88.2)	.071
	Accuracy, % (95% CI)	54.5 (36.4–71.9)	57.6 (39.2–74.5)	90.6 (75.0–98.0)	90.3 (74.2–98.0)	<.001
Stylet retraction	Sensitivity, % (95% CI)	65.4 (44.3–82.8)	76.7 (57.7–90.1)	92.6 (75.7–99.1)	100 (86.8–100)	.003
	Specificity, % (95% CI)	100 (59.0–100)	100 (29.2–100)	100 (47.8–100)	100 (47.8–100)	.999
	PPV, % (95% CI)	100 (80.5–100)	100 (85.2–100)	100 (86.3–100)	100 (86.8–100)	.999
	NPV, % (95% CI)	43.8 (19.8–70.1)	30.0 (6.7–65.2)	71.4 (29.0–96.3)	100 (47.8–100)	.045
	Accuracy, % (95% CI)	72.7 (54.5–86.7)	78.8 (61.1–91.0)	93.8 (79.2–99.2)	100 (88.8–100)	.005
Suction	Sensitivity, % (95% CI)	70.4 (49.8–86.2)	86.7 (69.3–96.2)	92.6 (75.7–99.1)	100 (86.8–100)	.023
	Specificity, % (95% CI)	100 (54.1–100)	100 (29.2–100)	100 (47.8–100)	100 (47.8–100)	.999
	PPV, % (95% CI)	100 (82.4–100)	100 (86.8–100)	100 (86.3–100)	100 (86.8–100)	.999
	NPV, % (95% CI)	42.9 (17.7–71.1)	42.9 (9.9–81.6)	71.4 (29.0–96.3)	100 (47.8–100)	.107
	Accuracy, % (95% CI)	75.8 (57.7–88.9)	87.9 (71.8–96.6)	93.8 (79.2–99.2)	100 (88.8–100)	.014
Pancreatic neoplasia only						
All techniques	Sensitivity, % (95% CI)	64.1 (52.4–74.7)	74.4 (64.2–83.1)	93.8 (86.2–98.0)	98.7 (93.1–100)	<.001
No suction	Sensitivity, % (95% CI)[a]	53.8 (33.4–73.4)	60.0 (40.6–77.3)	96.3 (81.0–99.9)	96.2 (80.4–99.9)	<.001
Stylet retraction	Sensitivity, % (95% CI)[a]	65.4 (44.3–82.8)	76.7 (57.7–90.1)	92.6 (75.7–99.1)	100 (86.8–100)	.003
Suction	Sensitivity, % (95% CI)[a]	73.1 (52.2–88.4)	87.7 (69.3–96.2)	92.6 (75.7–99.1)	100 (86.8–100)	.022

NPV, negative predictive value; PPV, positive predictive value.

[a]Sensitivity for the diagnosis of pancreatic neoplasia was as follows: Menghini-tip vs franseen: sensitivity was significantly higher for the franseen needle for no suction ($P = .001$). However, there was no significant different between the franseen and Menghini-tip needles for suction ($P = .467$) and stylet retraction techniques ($P = .100$). Menghini-tip vs fork-tip: sensitivity was significantly higher for the fork-tip needle for no suction ($P = .001$) and stylet retraction ($P = .008$). However, there was no significant difference between the fork-tip and Menghini-tip needles for suction ($P = .053$).

sensitivities of 73.1% for the reverse-bevel and 87.7% for the Menghini-tip needles, when applying suction (Table 4).

On reverse-stepwise multivariate logistic regression analysis, the use of the franseen needle, fork-tip needle, not applying suction, use of stylet retraction, and pancreatic mass located in the body/tail regions of the pancreas were associated with high cellularity (Table 3).

Summary Analysis of the Performances of Individual Fine-Needle Biopsy Needles

A summary analysis of the performance of individual FNB needles for a single pass is shown in Tables 2 and 4, and Supplementary Table 3. A detailed description of the performances of individual FNB needles is included in the Supplementary Appendix. The recommended procedural technique for each FNB

Table 5. Recommended Procedural Techniques to Achieve Optimal Overall Outcomes for Each FNB Needle Type

Needle type	Recommended technique: Best cellularity	Best diagnostic accuracy	Best overall outcome (specimen)
Reverse-bevel	Stylet retraction = suction	Suction	Suction
Menghini-tip	No suction = stylet retraction = suction	Suction	Suction
Franseen	Stylet retraction	Stylet retraction = suction	Stylet retraction
Fork-tip	No suction = stylet retraction	Stylet retraction = suction	Stylet retraction

FNB, fine-needle biopsy.

needle to achieve the optimal overall outcome is shown in Table 5.

Discussion

The present study confirms our hypothesis that outcomes of EUS-FNB of pancreatic masses are reliant on tailoring procedural technique to the type of needle used for tissue sampling. Overall, the franseen and fork-tip needles yielded higher cellularity and achieved a high diagnostic accuracy exceeding 90% on a single pass, which was significantly higher compared with other FNB needles.

Prior studies have shown that the presence of an onsite cytopathologist improves the operating characteristics of EUS-FNA of pancreatic masses by yielding a diagnostic sensitivity of 88% to 95% compared with only 80% or less in the absence of a cytopathologist.[4] However, in the United States, ROSE is available only in select centers and seldom available outside the country. Although the use of a cell block specimen processing technique eliminated the need for ROSE, the diagnostic accuracy for specimens procured using an FNA needle was only 71.9%.[2] Although the FNB needle was developed to overcome this limitation, several studies including a recent meta-analysis, did not show a significant improvement over FNA needles.[18,23] However, the majority of these studies evaluated older-generation, reverse-bevel needles and not the newer-generation FNB needles.[24] In the present study, the diagnostic adequacy and accuracy of biopsy samples procured using the franseen and fork-tip needles exceeded 90%.

The present study reinforces observations from prior randomized trials on EUS-guided tissue acquisition: the use of suction increases sample bloodiness.[2,3] However, although bloodiness is a detriment to the diagnostic performance of ROSE, it does not appear to significantly impact operating characteristics of biopsy samples processed as histologic specimens. Although not applying suction had no major impact on the performance of both franseen and fork-tip needles, it significantly decreased the diagnostic performance of the other 2 needles and only the application of suction improved the operating characteristics of both reverse-bevel and Menghini-tip needles. To optimize cellularity and yield a high diagnostic accuracy, we found stylet retraction to be the most optimal sampling technique for both the franseen and fork-tip needles.

What lessons can we learn from this randomized trial that can advance the role of EUS-guided tissue acquisition in pancreatic cancer? First, tissue acquisition using the franseen or fork-tip needle precludes the need for ROSE because histologic specimen processing yields an overall diagnostic accuracy and sensitivity for the detection of neoplasia that exceeds 90%. Second, the most optimal outcomes for biopsy samples can be achieved by tailoring the sampling technique to the needle type. We recommend stylet retraction during sampling for the franseen and fork-tip needles and applying suction to the Menghini-tip needle to achieve optimal outcomes. Third, although studies on EUS-FNA of pancreatic cancer suggest that a minimum of 4 passes is required to achieve a diagnostic sensitivity of more than 90%,[25] in the present study, similar diagnostic sensitivities could be achieved with just a single pass when using the franseen or fork-tip needles. In a recent prospective study, pancreatic adenocarcinoma organoids were isolated successfully with just a single pass during EUS-FNB.[26] For centers that rely on the reverse-bevel and Menghini-tip needles, we recommend performing 2 to 3 passes using suction to achieve optimal outcomes. Fourth, specimens procured using the franseen and fork-tip needles are of high cellularity and represent histology-grade tissue.[6] By using a basic science digital software that categorized specimens into individual tissue components, we observed that both needles yielded more tissue with retained histologic architecture, which is relevant for evaluating diseases such as autoimmune or chronic pancreatitis, which can mimic well-differentiated pancreatic cancer. Furthermore, in this study, desmoplastic fibrosis was seen in 92% and 91% of study subjects with pancreatic adenocarcinoma who underwent sampling using the franseen and fork-tip needles, respectively, compared with only 42% and 47% with the Menghini-tip and reverse-bevel needles, respectively ($P < .001$) (Supplementary Figure 4). Desmoplasia is a cellular reaction to the neoplastic process but is difficult to acquire with standard bevel needles because it requires tissue "coring." This is relevant in clinical investigations because patients with activated stroma tend to have a poor prognosis and are less responsive to neoadjuvant therapy because the desmoplastic stromal proliferation inhibits delivery of chemotherapeutic agents and vascular penetration.[27] Because the majority of patients with pancreatic cancer do not undergo surgery, FNBs also can be used to develop ex vivo organoids as a testbed for therapeutic agents. Consequently, these findings at pathology may have therapeutic implications for delivery of oncologic care. Finally, by conclusively establishing the definitive needle and definitive technique that can yield a definitive diagnosis in the majority of patients, we believe that our findings may enable standardization of the practice of EUS-guided tissue acquisition in pancreatic cancer.

There were a few limitations to this study. First, we only evaluated patients with pancreatic mass lesions and hence the findings may not be applicable to patients with mass lesions in other organs. Second, because it was not possible to blind the endosonographers performing the procedure, the possibility of bias cannot be eliminated. However, this limitation is likely to be minimal because the pathologists and study coordinators were blinded to the type of FNB needle and technique used for tissue sampling. Third, in this study, the information on the presence of visible core tissue in specimen containers on

gross inspection was not collected and therefore its relationship with the degree of cellularity cannot be elucidated. Finally, because the study was performed at a tertiary referral center by experienced endosonographers, it is possible that the outcomes may not be generalizable to less experienced hands.

In conclusion, in patients with suspected pancreatic cancer, a highly cellular specimen with a diagnostic accuracy of more than 90% can be achieved by performing a single biopsy using the franseen or fork-tip FNB needle, adopting the recommended sampling technique.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2020.06.042.

References

1. Lee KY, Cho HD, Hwangbo Y, et al. Efficacy of 3 fine-needle biopsy techniques for suspected pancreatic malignancies in the absence of an on-site cytopathologist. Gastrointest Endosc 2019;89:825–831.
2. Bang JY, Navaneethan U, Hasan MK, et al. Endoscopic ultrasound-guided specimen collection and evaluation techniques affect diagnostic accuracy. Clin Gastroenterol Hepatol 2018;16:1820–1828.
3. Wallace MB, Kennedy T, Durkalski V, et al. Randomized controlled trial of EUS-guided fine needle aspiration techniques for the detection of malignant lymphadenopathy. Gastrointest Endosc 2001;54:441–447.
4. Hebert-Magee S, Bae S, Varadarajulu S, et al. The presence of a cytopathologist increases the diagnostic accuracy of endoscopic ultrasound-guided fine needle aspiration cytology for pancreatic adenocarcinoma: a meta-analysis. Cytopathology 2013;24:159–171.
5. Varadarajulu S, Tamhane A, Eloubeidi MA. Yield of EUS-guided FNA of pancreatic masses in the presence or the absence of chronic pancreatitis. Gastrointest Endosc 2005;62:728–736.
6. Bang JY, Hebert-Magee S, Navaneethan U, et al. EUS-guided fine needle biopsy of pancreatic masses can yield true histology. Gut 2018;67:2081–2084.
7. Bang JY, Hebert-Magee S, Navaneethan U, et al. Randomized trial comparing the Franseen and Fork-tip needles for EUS-guided fine-needle biopsy sampling of solid pancreatic mass lesions. Gastrointest Endosc 2018;87:1432–1438.
8. Bang JY, Kirtane S, Krall K, et al. In memoriam: Fine-needle aspiration, birth: fine-needle biopsy: the changing trend in endoscopic ultrasound-guided tissue acquisition. Dig Endosc 2019;31:197–202.
9. Bang JY, Hawes R, Varadarajulu S. A meta-analysis comparing ProCore and standard fine-needle aspiration needles for endoscopic ultrasound-guided tissue acquisition. Endoscopy 2016;48:339–349.
10. Jovani M, Abidi WM, Lee LS. Novel fork-tip needles versus standard needles for EUS-guided tissue acquisition from solid masses of the upper GI tract: a matched cohort study. Scand J Gastroenterol 2017;52:784–787.
11. Kandel P, Tranesh G, Nassar A, et al. EUS-guided fine needle biopsy sampling using a novel fork-tip needle: a case-control study. Gastrointest Endosc 2016;84:1034–1039.
12. Tempero MA, Malafa MP, Chiorean EG, et al. Pancreatic adenocarcinoma, version 1.2019. J Natl Compr Canc Netw 2019;17:202–210.
13. Roy A, Kim M, Hawes R, et al. Changing trends in tissue acquisition in malignant pancreatic neoplasms. J Gastroenterol Hepatol 2016;31:501–505.
14. de Paz P, Mata-Campuzano M, Tizado EJ, et al. The relationship between ram sperm head morphometry and fertility depends on the procedures of acquisition and analysis used. Theriogenology 2011;76:1313–1325.
15. Khatri P, Choudhury M, Jain M, et al. Role of morphometry in the cytological differentiation of benign and malignant thyroid lesions. J Cytol 2017;34:1–4.
16. Kashyap A, Jain M, Shukla S, et al. Study of nuclear morphometry on cytology specimens of benign and malignant breast lesions: a study of 122 cases. J Cytol 2017;34:10–15.
17. Cotton PB, Eisen GM, Aabakken L, et al. A lexicon for endoscopic adverse events: report of an ASGE workshop. Gastrointest Endosc 2010;71:446–454.
18. Strand DS, Jeffus SK, Sauer BG, et al. EUS-guided 22-gauge fine-needle aspiration versus core biopsy needle in the evaluation of solid pancreatic neoplasms. Diagn Cytopathol 2014;42:751–758.
19. Alatawi A, Beuvon F, Grabar S, et al. Comparison of 22G reverse-beveled versus standard needle for endoscopic ultrasound-guided sampling of solid pancreatic lesions. United European Gastroenterol J 2015;3:343–352.
20. Witt BL, Adler DG, Hilden K, et al. A comparative needle study: EUS-FNA procedures using the HD ProCore(™) and EchoTip(®) 22-gauge needle types. Diagn Cytopathol 2013;41:1069–1074.
21. Othman MO, Abdelfathah MM, Padilla O, et al. The cellularity yield of three different 22-gauge endoscopic ultrasound fine needle aspiration needles. Diagn Cytopathol 2017;45:426–432.
22. Sterlacci W, Sioulas AD, Veits L, et al. 22-gauge core vs 22-gauge aspiration needle for endoscopic ultrasound-guided sampling of abdominal masses. World J Gastroenterol 2016;22:8820–8830.
23. Facciorusso A, Wani S, Triantafyllou K, et al. Comparative accuracy of needle sizes and designs for EUS tissue sampling of solid pancreatic masses: a network meta-analysis. Gastrointest Endosc 2019;90:893–903.
24. Bang JY, Varadarajulu S. Equal efficacy of FNA and fine-needle biopsy needles for EUS-guided tissue acquisition: really? Gastrointest Endosc 2019;90:904–905.
25. Mohamadnejad M, Mullady D, Early DS, et al. Increasing number of passes beyond 4 does not increase sensitivity of detection of pancreatic malignancy by endoscopic ultrasound-guided fine-needle aspiration. Clin Gastroenterol Hepatol 2017;15:1071–1078.
26. LaComb JF, Plenker D, Tiriac H, et al. Single-pass vs 2-pass endoscopic ultrasound-guided fine-needle biopsy sample collection for creation of pancreatic adenocarcinoma organoids. Clin Gastroenterol Hepatol 2020. Epub ahead of print. Q12
27. Jacobetz MA, Chan DS, Neesse A, et al. Hyaluronan impairs vascular function and drug delivery in a mouse model of pancreatic cancer. Gut 2013;62:112–120.

Reprint requests
Address requests for reprints to: Shyam Varadarajulu, MD, Center for Interventional Endoscopy, AdventHealth Orlando, 601 East Rollins Street, Orlando, Florida 32803. e-mail: svaradarajulu@yahoo.com; fax: (407) 303-2585.

Acknowledgments
CRediT Authorship Contributions: Ji Young Bang (Formal analysis: Lead; Investigation: Equal; Methodology: Equal; Writing – original draft: Equal; Writing – review & editing: Equal); Konrad Krall (Data curation: Equal; Investigation: Supporting; Visualization: Supporting; Writing – review & editing: Supporting); Nirag Jhala (Methodology: Supporting; Writing – review & editing: Supporting); Charanjeet Singh (Investigation: Supporting; Supervision: Supporting; Writing – review & editing: Supporting); Mohamedtaki Tejani (Supervision: Supporting; Writing – review & editing: Supporting); Juan Pablo Arnoletti (Supervision: Supporting; Writing – review & editing: Supporting); Udayakumar Navaneethan (Investigation: Supporting; Writing – review & editing: Supporting); Robert Hawes (Investigation: Supporting; Supervision: Supporting; Writing – review & editing: Supporting); Shyam Varadarajulu, MD (Conceptualization: Lead; Investigation: Lead; Methodology: Lead; Supervision: Lead; Writing – original draft: Lead; Writing – review & editing: Equal).

Conflicts of interest
These authors disclose the following: Ji Young Bang has served as a consultant for Olympus America, Inc, and Boston Scientific Corporation; Shyam Varadarajulu has served as a consultant for Boston Scientific Corporation, Olympus America, Inc, Covidien, and Creo Medical; and Robert Hawes has served as a consultant for Boston Scientific Corporation, Olympus America, Inc, Covidien, Creo Medical, Nine Points Medical, and Cook Medical. The remaining authors disclose no conflicts.

Supplementary Materials and Methods

Description of Fine-Needle Biopsy Needle Tip Geometries

Reverse-bevel. The 22G Echotip Procore (Cook Endoscopy) needle has a 5.2F shaft, a core trap of 2 mm, and a reverse-bevel length of 5.9 mm (Supplementary Figure 1*A*). The reverse-bevel at the side hole located just distal to the needle tip enhances suctioning of the tissue during to-and-fro movement inside the mass lesion.

Menghini-tip. The 22G EZ Shot 3 Plus (Olympus America) Menghini needle tip design features sharp, continuous cutting edges to cleanly cut tissue specimens while preserving the cellular architecture (Supplementary Figure 1*B*).

Franseen. The 22G Acquire (Boston Scientific Corporation) needle design has a crown-tip with 3 symmetric surfaces that manifest as 3 cutting edges (Supplementary Figure 1*C*). This unique geometry contributes to a longer insertion length and the area at the crown tip that facilitates greater tissue acquisition.

Fork-tip. The 22G SharkCore (Medtronic Corporation) needle has a second sharp tip on the opposite side of the lumen with the aim of improving tissue capture (Supplementary Figure 1*D*).

Procedural Technique

Description of sampling maneuvers. *Suction.* In the suction technique, after puncturing the pancreatic mass and removing the stylet, 20 mL of dry negative suction was applied before fanning and suction was released before removal of the needle.

Stylet retraction. In the stylet retraction technique, sampling was performed adopting the fanning maneuver, with simultaneous minimal negative pressure provided by retracting the needle stylet slowly and continuously to half of the length of the stylet.

No suction. In the no suction technique, sampling was performed using the fanning technique (4 strokes at 4 locations within the mass), without applying suction or retracting the stylet.

Tissue preparation and histologic assessment. The biopsy samples were submitted to the laboratory in 10% formalin, with each pass performed using the 3 different sampling maneuvers placed in separate containers. Once in the laboratory, the contents of the specimen container were poured through filter paper to capture the tissue cores and fragments. The filter paper then was folded around the cores and fragments. The folded filter then was placed into a cassette and submitted in formalin for processing, imbedding, sectioning, and mounting on slides. The completed slides were stained using the H&E stain process.

The presence of core tissue, blood, and crush artifact from tissue acquisition were recorded. The grading of cellularity was defined as low, intermediate, or high: low cellularity was defined as the proportion of core tissue to total specimen area of 0% to 10%, intermediate cellularity was defined as 11% to 50%, and high cellularity was defined as more than 50%.[1] Specimen bloodiness was categorized on the basis of percentage of blood in the microscopic field: mild, less than 33%; moderate, 33% to 66%; and severe, more than 66%.[2] Crush artifact was defined as areas of distortion of histologic tissue architecture during specimen processing.[3]

Immunohistochemistry studies for evaluation of morphologically challenging lesions and molecular profiling for tailoring chemotherapy regimens were performed when required.

Results

Performances of the Individual Fine-Needle Biopsy Needles: Single Pass

Reverse-bevel. A maximum diagnostic adequacy of 84.8% and a median cellularity of 23.4% could be achieved by applying suction. Specimen bloodiness on average was highest for the reverse-bevel needle irrespective of the sampling technique. A maximum diagnostic sensitivity and accuracy of 70.4% and 75.8%, respectively, could be achieved when applying suction, however, it was the lowest among all FNB needles. Therefore, the most optimal overall outcome, taking into consideration cellularity and diagnostic accuracy, could be achieved only by applying suction.

Menghini-tip. A maximum diagnostic adequacy of 100% could be achieved by applying suction, and a maximum median cellularity of 30.3% could be achieved by the stylet retraction technique. A maximum diagnostic sensitivity and accuracy of 86.7% and 87.9%, respectively, could be achieved with suction. Therefore, the most optimal overall outcome, taking into consideration cellularity and diagnostic accuracy, could be achieved by applying suction.

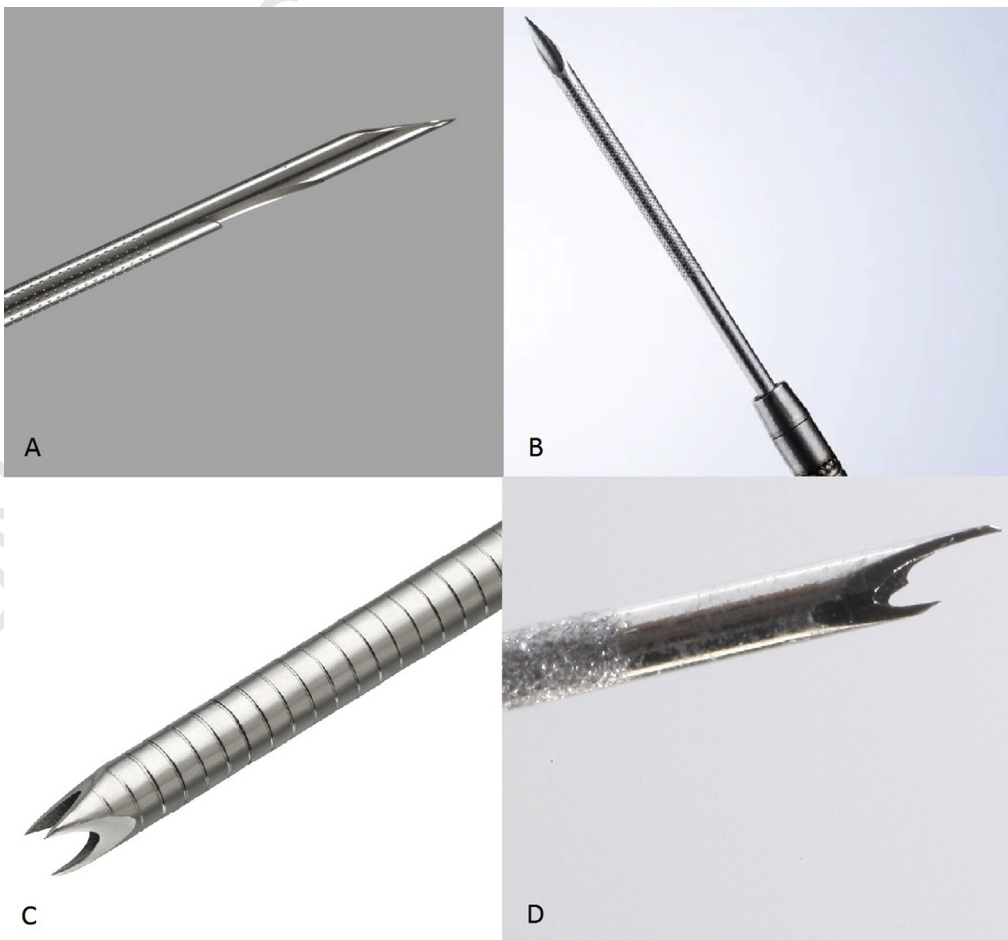
Franseen. A maximum diagnostic adequacy of 93.8% to 100% could be achieved using any of the 3 sampling techniques, and a maximum median cellularity of 73.1% was achieved by stylet retraction. The specimen bloodiness was classified as high in 43.8% of biopsy samples when applying suction, but was classified as high in only 12.5% to 15.6% for the other 2 techniques. A maximum diagnostic sensitivity and accuracy of 92.6% and 93.8%, respectively, could be achieved using stylet retraction or by applying suction. Therefore, the most optimal overall outcome, taking into consideration cellularity and diagnostic accuracy, could be achieved by the stylet retraction technique.

Fork-tip. A maximum diagnostic adequacy of 93.5% to 100% could be achieved using any of the 3 sampling techniques and a median cellularity of more than 95% was achieved by not applying suction; the median cellularity for the stylet retraction technique was the second highest at 55.5%. Specimen bloodiness was classified as high in 45% of

biopsy samples when applying suction, but was classified as high in less than 23% of the biopsy samples for other techniques. A maximum diagnostic sensitivity and accuracy of 100% could be achieved in the biopsy samples by stylet retraction or by applying suction. Therefore, the most optimal overall outcome, taking into consideration cellularity and diagnostic accuracy, could be achieved by the stylet retraction technique.

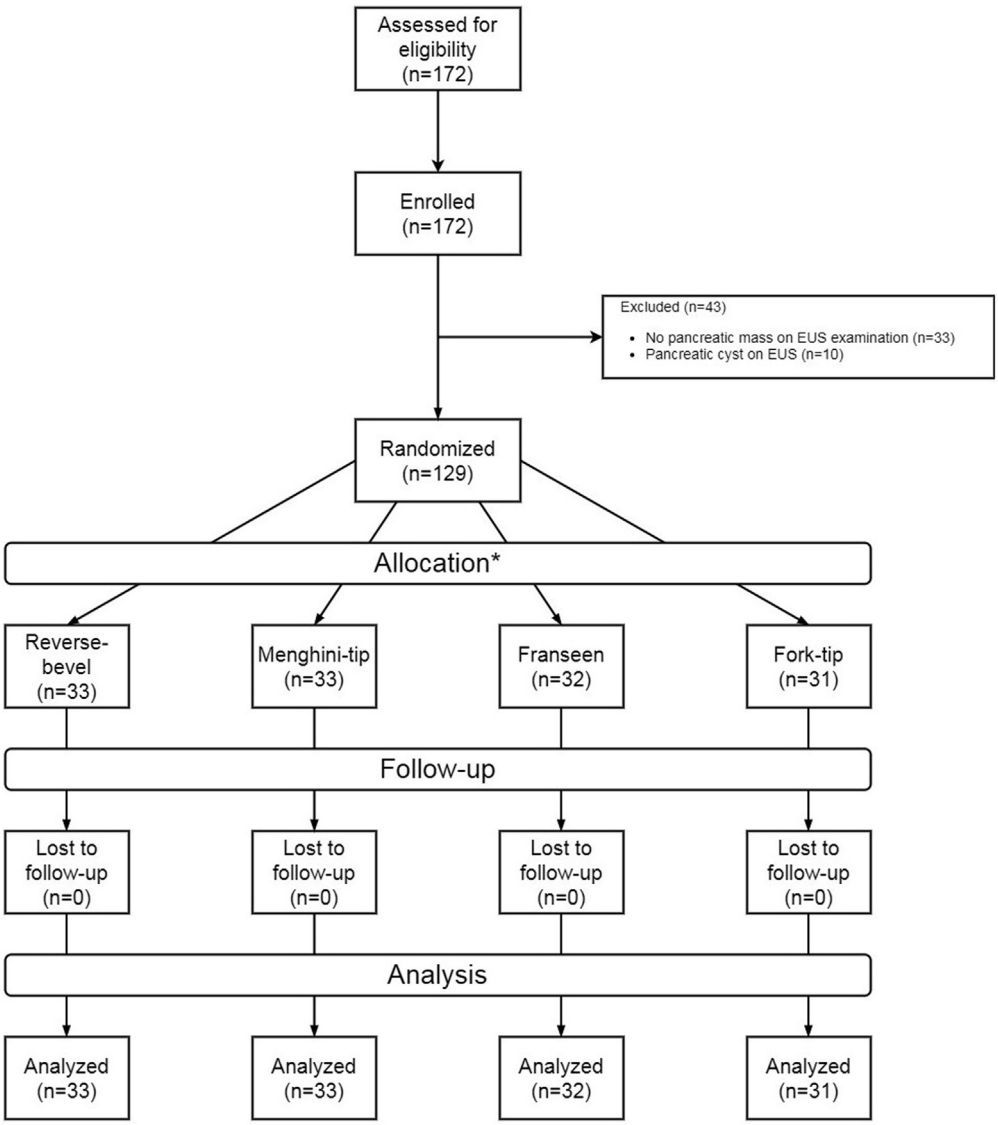
References

1. Jhala N, Jhala D. Definitions in tissue acquisition: core biopsy, cell block, and beyond. Gastrointest Endosc Clin N Am 2014; 24:19–27.
2. Varadarajulu S, Bang JY, Holt BA, et al. The 25-gauge EUS-FNA needle: good for on-site but poor for off-site evaluation? Results of a randomized trial. Gastrointest Endosc 2014;80:1056–1063.
3. Chatterjee S. Artefacts in histopathology. J Oral Maxillofac Pathol 2014;18:S111–S116.

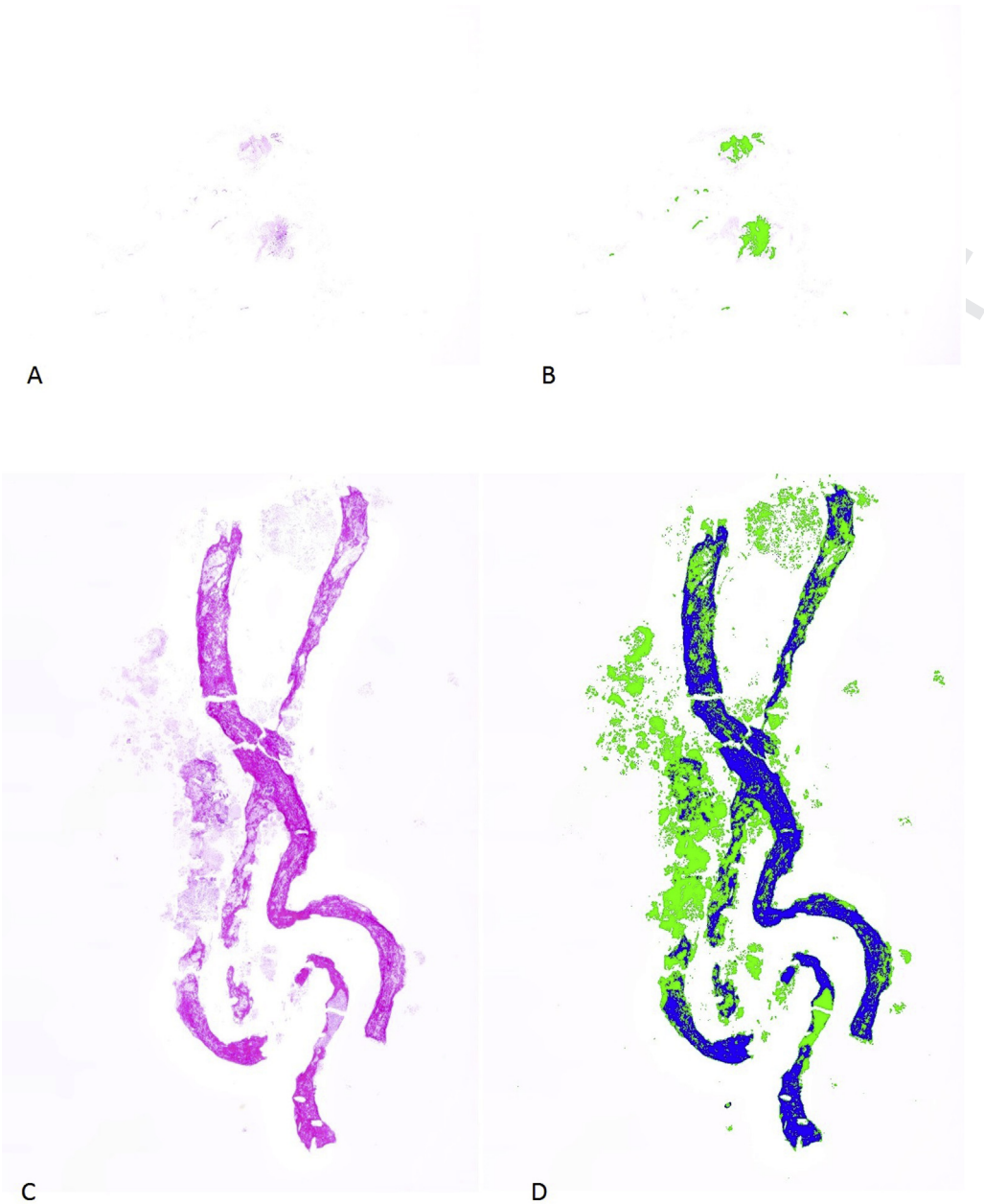


web 4C/FPO

Supplementary Figure 1. Needle-tip designs of the (*A*) reverse-bevel, (*B*) Menghini-tip, (*C*) franseen, and (*D*) fork-tip needles.

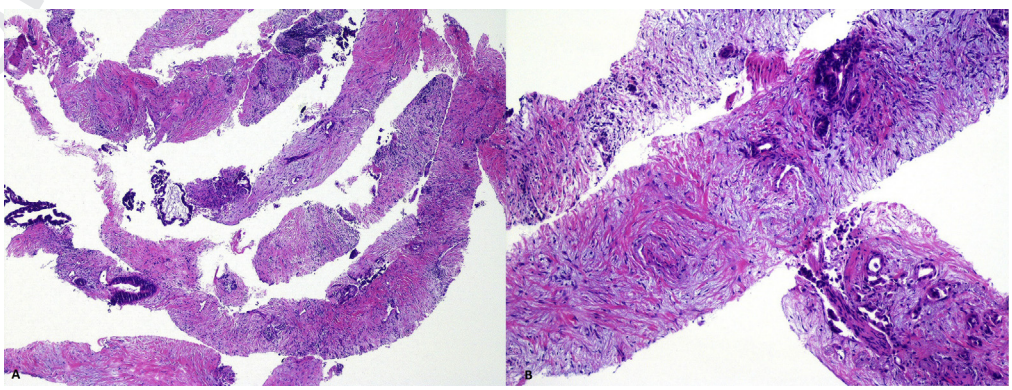


Supplementary Figure 2. The CONSORT flow diagram. EUS, endoscopic ultrasound. Q13



Supplementary Figure 3. Image showing H&E staining (*left panels*) of pancreatic adenocarcinoma (20×) with a corresponding digital image (*right panels*) showing adenocarcinoma with pancreatic acinar and ductal sells, blood, fibrosis, and crush artifact for (*A* and *B*) reverse-bevel and (*C* and *D*) Menghini-tip needles. In the digital images, core tissue is highlighted in green, blood is highlighted in blue, and crush artifact is highlighted in red. Q14

web 4C/FPO



Supplementary Figure 4. H&E staining of pancreatic adenocarcinoma of (A) 40× and (*B*) 100×, with the specimen showing desmoplastic fibrosis and malignant ductal epithelium.

web 4C/FPO

Supplementary Table 1. Quantity of Crush Artifact and Presence of Blood for Each Technique for a Single Pass, With Comparison Between the Needles

	Reverse-bevel (n = 33)	Menghini-tip (n = 33)	Franseen (n = 32)	Fork-tip (n = 31)	*P* value
Technique: no suction					
Quantity of crush artifact, %					
Mean (SD)	0.013 (0.056)	0.014 (0.063)	0.064 (0.25)	0.50 (2.4)	
Median	0	0	0	0	.741
IQR	0–0	0–0	0–0	0–0	
Range	0–0.29	0–0.35	0–1.4	0–13.2	
Specimen bloodiness, n (%)					
Low	11 (33.3)	19 (57.6)	20 (62.5)	23 (74.2)	<.001
Moderate	4 (12.1)	0	8 (25.0)	2 (6.5)	
High	18 (54.5)	14 (42.4)	4 (12.5)	6 (19.4)	
Technique: stylet retraction					
Quantity of crush artifact, %					
Mean (SD)	0.010 (0.040)	0.0055 (0.031)	0.13 (0.39)	0.30 (1.3)	
Median	0	0	0	0	.236
IQR	0–0	0–0	0–0.085	0–0	
Range	0–0.20	0–0.18	0–2.0	0–7.4	
Specimen bloodiness, n (%)					
Low	8 (24.2)	10 (30.3)	17 (53.1)	14 (45.2)	.002
Moderate	4 (12.1)	8 (24.2)	10 (31.3)	10 (32.3)	
High	21 (63.6)	15 (45.5)	5 (15.6)	7 (22.6)	
Technique: suction					
Quantity of crush artifact, %					
Mean (SD)	0.36 (1.9)	0.029 (0.092)	0.052 (0.13)	0.081 (0.33)	
Median	0	0	0	0	.874
IQR	0–0	0–0	0–0.03	0–0	
Range	0–10.9	0–0.44	0–0.63	0–1.8	
Specimen bloodiness, n (%)					
Low	8 (24.2)	4 (12.1)	7 (21.9)	8 (25.8)	.332
Moderate	5 (15.2)	7 (21.2)	11 (34.4)	9 (29.0)	
High	20 (60.6)	22 (66.7)	14 (43.8)	14 (45.2)	

IQR, interquartile range.

Supplementary Table 2. Quantity of Crush Artifact and Presence of Blood for Each Needle Type for a Single Pass, With Comparison Between the Techniques

	No suction	Stylet retraction	Suction	*P* value
Needle: reverse-bevel (n = 33)				
Quantity of crush artifact, %				
Mean (SD)	0.013 (0.056)	0.010 (0.040)	0.36 (1.9)	
Median	0	0	0	.792
IQR	0–0	0–0	0–0	
Range	0–0.29	0–0.20	0–10.9	
Specimen bloodiness, n (%)				
Low	11 (33.3)	8 (24.2)	8 (24.2)	.901
Moderate	4 (12.1)	4 (12.1)	5 (15.2)	
High	18 (54.5)	21 (63.6)	20 (60.6)	
Needle: Menghini-tip (n = 33)				
Quantity of crush artifact, %				
Mean (SD)	0.014 (0.063)	0.0055 (0.031)	0.029 (0.092)	
Median	0	0	0	.683
IQR	0–0	0–0	0–0	
Range	0–0.35	0–0.18	0–0.44	
Specimen bloodiness: n (%)				
Low	19 (57.6)	10 (30.3)	4 (12.1)	<.001
Moderate	0	8 (24.2)	7 (21.2)	
High	14 (42.4)	15 (45.5)	22 (66.7)	
Needle: franseen (n = 32)				
Quantity of crush artifact, %				
Mean (SD)	0.064 (0.25)	0.13 (0.39)	0.052 (0.13)	
Median	0	0	0	.632
IQR	0–0	0–0.085	0–0.030	
Range	0–1.4	0–2.0	0–0.63	
Specimen bloodiness, n (%)				
Low	20 (62.5)	17 (53.1)	7 (21.9)	.005
Moderate	8 (25.0)	10 (31.3)	11 (34.4)	
High	4 (12.5)	5 (15.6)	14 (43.7)	
Needle: fork-tip (n = 31)				
Quantity of crush artifact, %				
Mean (SD)	0.50 (2.4)	0.30 (1.3)	0.081 (0.33)	
Median	0	0	0	.971
IQR	0–0	0–0	0–0	
Range	0–13.2	0–7.4	0–1.8	
Specimen bloodiness, n (%)				
Low	23 (74.2)	14 (45.2)	8 (25.8)	.002
Moderate	2 (6.4)	10 (32.3)	9 (29.0)	
High	6 (19.4)	7 (22.6)	14 (45.2)	

IQR, interquartile range.

Supplemental Table 3. Procedure Outcomes for Biopsy Samples for Each Needle Type for a Single Pass, With Comparison Between Techniques

	No suction	Stylet retraction	Suction	*P* value
Needle: reverse-bevel (n = 33)				
Diagnostic adequacy, n (%)	21 (63.6)	27 (81.8)	28 (84.8)	.088
Cellularity, %				
Mean (SD)	25.8 (33.8)	31.5 (31.7)	31.2 (32.4)	
Median[a]	9.3	22.4	23.4	.235
IQR	0–41.0	6.8–38.4	7.2–50.3	
Range	0–100	0–100	0–100	
Low	17 (51.5)	10 (30.3)	13 (39.4)	.945[b]
Intermediate	8 (24.2)	16 (48.5)	12 (36.4)	
High[a]	8 (24.2)	7 (21.2)	8 (24.2)	
Crush artifact present, n (%)	2 (6.1)	3 (9.1)	5 (15.2)	.459
Needle: Menghini-tip (n = 33)				
Diagnostic adequacy, n (%)	22 (66.7)	29 (87.9)	33 (100)	.001
Cellularity, %				
Mean (SD)	31.6 (40.8)	34.7 (31.9)	29.5 (24.8)	
Median[c]	8.1	30.3	23.2	.216
IQR	0–77.7	4.1–49.5	10.9–40.9	
Range	0–100	0–100	1.0–93.8	
Low	18 (54.5)	10 (30.3)	8 (24.2)	.341[b]
Intermediate	5 (15.2)	15 (45.5)	20 (60.6)	
High[c]	10 (30.3)	8 (24.2)	5 (15.2)	
Crush artifact present, n (%)	2 (6.1)	1 (3.0)	5 (15.2)	.171
Needle: Franseen (n = 32)				
Diagnostic adequacy, n (%)	30 (93.8)	32 (100)	32 (100)	.130
Cellularity, %				
Mean (SD)	63.1 (36.9)	66.6 (30.9)	40.5 (31.3)	
Median[d]	70.3	73.1	34.5	.004
IQR	36.9–99.4	40.5–98.8	9.6–61.5	
Range	0–100	5.0–100	0.069–99.9	
Low	5 (15.6)	2 (6.2)	9 (28.1)	.029[b]
Intermediate	7 (21.9)	8 (25.0)	11 (34.4)	
High[d]	20 (62.5)	22 (68.8)	12 (37.5)	
Crush artifact present, n (%)	6 (18.8)	10 (31.3)	8 (25.0)	.513
Needle: fork-tip (n = 31)				
Diagnostic adequacy, n (%)	29 (93.5)	31 (100)	31 (100)	.130
Cellularity, %				
Mean (SD)	74.6 (36.8)	61.0 (31.6)	43.3 (30.8)	
Median[e]	96.5	55.5	41.3	.003
IQR	48.5–100	33.7–99.7	16.1–66.4	
Range	0–100	4.0–100	0.28–100	
Low	3 (9.7)	2 (6.5)	5 (16.1)	.004[b]
Intermediate	5 (16.1)	12 (38.7)	16 (51.6)	
High[e]	23 (74.2)	17 (54.8)	10 (32.3)	
Crush artifact present: n (%)	5 (16.1)	5 (16.1)	4 (12.9)	.919

IQR, interquartile range.

[a]Reverse-bevel needle: there was no significant difference in the median cellularity between no suction and stylet retraction ($P = .117$) and between no suction and suction techniques ($P = .175$). There was also no significant difference in high vs low/intermediate cellularity between no suction and stylet retraction ($P = .769$) and between no suction and suction ($P = .999$) techniques.

[b]For comparison of degree of cellularity, the *P* value is for comparison of low-/intermediate-cellularity vs high-cellularity biopsy samples.

[c]Menghini-tip needle: there was no significant difference in the median cellularity between no suction and stylet retraction ($P = .175$) and between no suction and suction techniques ($P = .093$). There was also no significant difference in high vs low/intermediate cellularity between no suction and stylet retraction ($P = .580$) and between no suction and suction ($P = .142$) techniques.

[d]Franseen needle: the median cellularity was significantly higher for no suction compared with suction ($P = .010$), and significantly higher for stylet retraction compared with suction ($P = .001$). The proportion of high vs low/intermediate cellularity also was significantly higher for no suction compared with suction ($P = .046$) and higher for stylet retraction compared with suction ($P = .012$).

[e]Fork-tip needle: the median cellularity was significantly higher for no suction compared with suction ($P = .001$), however, no significant difference was observed for no suction compared with stylet retraction ($P = .114$). The proportion of high vs low/intermediate cellularity also was significantly higher for no suction compared with suction ($P = .001$), however, no significant difference was observed between no suction and stylet retraction ($P = .111$).